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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 09/920,571	Applicant(s) LASKEN ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 July 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,5-9,14,20,22-25,27,29,35-39,41,42,44-49,51-53,55-59 and 69-84* is/are pending in the application.

4a) Of the above claim(s) *74,76-82 and 84* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,5-9,14,20,22-25,27,29,35-39,41,42,44-49,51-53,55-59,69-73,75 and 83* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7/21/08*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. This office action is in response to an amendment filed July 2, 2008. Claims 1, 5-9, 14, 20, 22-25, 27, 29, 35-39, 41, 42, 44-49, 51-53, 55-59 and 69-84 were previously pending, with claims 74, 76-82 and 84 withdrawn from consideration. Applicants amended claims 20, 22 and 23. Claims 1, 5-9, 14, 20, 22-25, 27, 29, 35-39, 41, 42, 44-49, 51-53, 55-59, 69-73, 75 and 83 will be examined.

2. Applicants' amendments overcame the rejection of claims 20, 22 and 23 under 35 U.S.C. 112, second paragraph. All other previously presented rejections are maintained for reasons given in the "Response to Arguments" section below.

Information Disclosure Statement

3. The information disclosure statement (IDS) submitted on July 21, 2008 was filed after the mailing date of the non-final office action on March 17, 2008. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Response to Arguments

4. Applicant's arguments filed July 2, 2008 have been fully considered but they are not persuasive.

A) Regarding the claim interpretation, Applicants argue that examiner's conclusion that totally random primers are also degenerate primers is incorrect in view of Applicants' definition of a "degenerate primer" on page 6, lines 16-18. Let us look at the definition (page 6, lines 16-21):

"Degenerate refers to an oligonucleotide in which one or more of the nucleotide positions is occupied by more than one base, i.e., a mixture of oligonucleotides of defined length

in which one or more positions of an individual member of the mixture is occupied by more than one base selected at random from among more than one possibilities for that position."

Therefore, the definition of degenerate primers encompasses totally random primers because there is no requirement in the definition that the degenerate primer cannot have all of its positions occupied by randomly selected bases, i.e., random primers are a subset of degenerate primers. Let us illustrate this by an example. Let's assume that we want to create a set six-nucleotide degenerate primers. Therefore, according to the definition, <u>at least one</u> of the positions in such primers can be occupied by a random base. This means one, two, three, four, five and <u>six</u>. In the last case we get a set of random hexamers. To illustrate further, let us take a primer ATGCAT as an example, and let us denote by N a random position. A set of degenerate hexamer primers with one random position will have the following sequences: ATGCAN, ATGCNT, ATGNAT, ATNCAT, ANGCAT and NTGCAT. A set of primers with two random positions will have sequences: ATGCNN, ATNNAT, NNGCAT, NTGCAN, NTGCNT, NTGNCAT, etc. A set of primers with three different random positions will have the following sequences: ATGNNN, NNNCAT, NTGCNN, NTGNNT, NTNNAT, etc. A set of degenerate primers with four different random positions will contain the following sequences: ATNNNN, NNNNAT, NTGNNN, NTNNNT, ANNNAT, etc. A set of degenerate primers with five different random positions will have the following sequences: ANNNNN, NNNNNT, NTNNNN, NNGNNN, etc. Finally, a set of primers with all six positions being random will have sequences NNNNNN, i.e., <u>we have arrived at a set of totally random hexamers</u>. Therefore, it is clear that Applicants' definition of degenerate primers encompasses totally random primers as a subset, or species, i.e., totally random primers are degenerate primers according to Applicants' definition.

Applicants further argue as follows (page 11 of the response, first paragraph):

"Furthermore, when viewed in context, Applicants' claims distinguish degenerate primers from random primers. For example, in claim 1, Applicants claim "A process for selectively amplifying nucleic acid sequences comprising contacting multiple single-stranded non-circular degenerate oligonucleotide primers ...."(emphasis added). One skilled in the art would understand that degenerate primers rather than totally or completely random primers would selectively amplify the target nucleic acid sequence. One skilled in the art would also understand that the use of totally or completely random primers would result in the random amplification, BUT NOT the selective amplification, of the target nucleic acid sequence. Therefore, in light of this discussion, Applicants respectfully request the Examiner withdraw this interpretation and adopt an intrepretation of degenerate primers that is consistent with Applicants' disclosure and the art."

If the above was true, one of skill in the art would be truly confused, because the paragraph cited below is present in the specification on page 20, lines 19-28:

"In one embodiment of the methods of the present invention, completely random primers are used for the amplification process, a particularly desirable process because the sequence of the ATC providing the template may not be known. Thus, any single stranded or duplex DNA circles can be readily used, with or without extensive purification, according to the methods disclosed herein. Thus, a major advantage of the use of random primers is that circular DNA targets of known or unknown sequence may be preferentially and selectively amplified from among a complex mixture of DNA molecules containing mixtures of both linear and circular DNA molecules." (emphasis added).

Therefore, unless the term "selectively amplifying" in claim 1 has a different meaning than "selectively amplified" in the above paragraph, Applicants contradict themselves. Further, claim 25 is drawn to specifically amplifying a target sequence with unknown sequence composition, which can be achieved using totally random primers. Finally, the term "selectively amplifying" has not been defined anywhere in the specification.

In view of the above evidence presented, examiner asserts that the interpretation of the term "degenerate primer" is proper and it is therefore maintained.

B) Regarding the rejection of claims 1, 5-8, 14, 20, 22, 23, 25, 27, 35, 38, 51, 55-58, 69, 70 and 83 under 35 U.S.C. 102(b) as being anticipated by Navarro et al., as evidenced by Kool and Oyama et al., Applicants argue the following:

i) Navarro et al. do not teach selective amplification, because they teach using random primers, which are not degenerate, and therefore the amplification cannot be selective.

This argument was addressed above.

ii) Only one reference should be used in a 102 rejection, unless, as stated by MPEP 2131.01, the additional reference provide either enablement, or explanation of terms, or shows characteristic not disclosed in the reference is inherent. Applicants argue that Kool et al. and Oyama et al. do not demonstrate that a characteristic not disclosed in Navarro et al. is inherent because examiner has not provided a basis in fact and/or technical reasoning to make the inherency assertion.

First, let us look at the facts. In Fig. 1 of Navarro et al. we have multiple primers annealed to a circular target, which primers are extended by a DNA polymerase. The target circles are 300-457 nucleotides long. Navarro et al. do not specifically teach that the resulting amplification products contain multiple copies of the target circle. Therefore Kool is relied upon to provide evidence that amplification of circular nucleic acids in the range of sizes from 15 to 15,000 nucleotides (which encompasses the circular RNAs of Navarro et al.) results in the production of multiple copies of the target sequence, which they state specifically in Fig. 1; col. 5, lines 51-67; col. 6, lines 1-33; col. 12, lines 58-67; col. 13, lines 1-19; with specific examples presented in col. 34, lines 53-67 and col. 35, lines 1-20. Further, Kool teaches that polymerases useful in the amplification process include Klenow fragment of DNA polymerase I and AMV reverse transcriptase (col. 13, lines 62-67), which are exactly the polymerases used by Navarro et

al. Therefore, Kool provides the missing inherent characteristic of the process described by Navarro et al. Finally, Oyama et al. was used to provide the missing inherent characteristic of the AMV reverse transcriptase, namely, that it does not possess the 3'->5' exonuclease activity. Therefore, contrary to Applicants' assertions, both references provide evidence for the inherent characteristics of the amplification reaction and AMV reverse transcriptase, respectively.

The rejection is maintained.

C) Regarding the rejection of claim 9 under 35 U.S.C. 103(a) over Navarro et al., as evidenced by Kool and Oyama et al., Applicants repeat their arguments about Navarro et al. not teaching selective amplification or degenerate primers and Kool and Oyama et al. being improperly used as supporting references.

These arguments were addressed above.

The rejection is maintained.

D) Regarding the rejection of claims 29, 38, 39, 41, 42, 44-47 and 59 under 35 U.S.C. 103(a) over Navarro et al., as evidenced by Kool and Oyama et al., in view of Skerra, Applicants repeat their arguments about Navarro et al. not teaching selective amplification or degenerate primers and Kool and Oyama et al. being improperly used as supporting references.

These arguments were addressed above.

Applicants further argue that Skerra does not teach selectively amplifying nucleic acids using multiple degenerate primers. However, Skerra is not used in the rejection to provide these limitations, but for the teaching of exonuclease-resistant primers.

The rejection is maintained.

E) Regarding the rejection of claims 35, 38, 44, 48 and 49 under 35 U.S.C. 103(a) over Navarro et al., as evidenced by Kool and Oyama et al., in view of Ott et al., Applicants repeat

their arguments about Navarro et al. not teaching selective amplification or degenerate primers and Kool and Oyama et al. being improperly used as supporting references.

These arguments were addressed above.

Applicants further argue that Ott et al. do not teach selectively amplifying nucleic acids using multiple degenerate primers. However, Ott et al. is not used in the rejection to provide these limitations, but for the teaching of phosphorothioate nucleotides in primers.

The rejection is maintained.

F) Regarding the rejection of claims 1, 5-9, 14, 20, 22-25, 27, 35, 38, 51, 55-58, 69-71, 75 and 83 under 35 U.S.C. 103(a) over Kool and Navarro et al., as evidenced by Oyama et al., Applicants argue the following:

i) Both references fail to teach selective amplification of nucleic acid sequences using multiple degenerate primers.

This argument was addressed above with respect to the Navarro et el. reference, which provides this teaching.

ii) There is no motivation to combine the methods of Navarro et al. and Kool, since Navarro et al. is only concerned with amplification of circular RNAs with unknown sequences using random primers, whereas Kool teaches amplification of circular sequences using specific primers. Therefore, using random hexamers in the amplification methods of Kool would be counterproductive and there is no expectation of success in achieving Kool's goals. Finally, using the random primer of Navarro et al. would alter a fundamental principle of operation of Kool, where known nucleotide sequences are amplified, therefore using random hexamers to amplify known sequences would not work.

First, contrary to Applicants' arguments, Kool teaches libraries of circular targets with random, i.e., unknown sequences (see, for example, Fig. 2 and Example 12). Then, since Kool teaches amplification of both DNA and RNA circular molecules, using the random primers of Navarro et al. would allow one of skill in the art to amplify circular molecules of unknown sequences with high efficiency. Therefore, one of skill in the art would understand that Navarro et al. provided a useful addition to the method of Kool, allowing amplification of circular molecules with unknown sequences, not of sequences already known, for which it would be obvious to one of skill in the art to use specific primers. Therefore the combination of references of Kool and Navarro et al. teach the claimed method.

The rejection is maintained.

G) Regarding the rejection of claims 29, 35-39, 44, 45, 48, 49 and 51-53 under 35 U.S.C. 103(a) over Kool and Navarro et al., as evidenced by Oyama et al., in view of Lizardi and Blanco et al., Applicants repeat their arguments concerning the combination of Kool and Navarro et al. references.

These arguments were addressed above.

The rejection is maintained.

H) Regarding the rejection of claims 29, 35, 38, 39, 41, 42, 44-47 and 59 under 35 U.S.C. 103(a) over Kool and Navarro et al., as evidenced by Oyama et al., in view of Skerra, Applicants repeat their arguments concerning the combination of Kool and Navarro et al. references.

These arguments were addressed above.

The rejection is maintained.

I) Regarding the rejection of claims 72 and 73 under 35 U.S.C. 103(a) over Kool and Navarro et al., as evidenced by Oyama et al., in view of Waggoner et al., Applicants repeat their arguments concerning the combination of Kool and Navarro et al. references.

These arguments were addressed above.

The rejection is maintained.

Claim Interpretation

5. Applicants defined the term "degenerate primers" on page 6, lines 16-21 as follows: "Degenerate refers to an oligonucleotide in which one or more of the nucleotide positions is occupied by more than one base, i.e., a mixture of oligonucleotides of defined length in which one or more positions of an individual member of the mixture is occupied by a base selected at random from among more than one possibilities for that position." Therefore totally random primers are also degenerate primers.

6. The term "selective amplification" has not been defined by Applicants, therefore any amplification is considered to be "selective".

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

8. Claims 1, 5-8, 14, 20, 22, 23, 25, 27, 35, 38, 51, 55-58, 69, 70 and 83 are rejected under 35 U.S.C. 102(b) as being anticipated by Navarro et al. (J. Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action), as evidenced by Kool (U.S. Patent No. 6,096,880 A; cited in the IDS) and Oyama et al. (Anal. Biochem., vol. 172, pp. 444-450, 1988).

Regarding claim 1, Navarro et al. teach a method of amplification comprising contacting multiple single stranded non-circular degenerate oligonucleotide primers (P1), one or more single stranded amplification target circles (ATCs), a DNA polymerase and multiple deoxynucleoside triphosphates (dNTPs), under conditions promoting said contacting, wherein each ATC hybridizes to a plurality of said P1 primers, wherein said conditions promote rolling circle replication of said amplification target circle by extension of the P1 primers to form multiple tandem sequence DNA (TS-DNA) products and wherein the TS-DNA is labeled during or following synthesis (Navarro et al. teach contacting multiple hexamer random (= degenerate) primers or degenerate 26 bp primers with a single stranded RNA circles in a presence of a DNA polymerase (AMV reverse transcriptase or Klenow fragment of DNA polymerase I) and multiple dNTPs under conditions promoting rolling circle replication by extension of the primers to form multiple TS-DNA products and wherein the TS-DNA is labeled following synthesis (Fig. 1; page 59, first paragraph; page 60, paragraphs 3-6; page 61, first paragraph; Fig. 2). Navarro et al. do not specifically teach that TS-DNA is obtained in the first step of the viral circles amplification. However, as evidenced by Kool, amplification of small circular DNA or RNA molecules in the range of 15 to 15,000 nucleotides in the presence of a primer and a DNA polymerase results in production of multiple copies of the circular target by rolling circle synthesis (Fig. 1; col. 5, lines 51-67; col. 6, lines 1-33; col. 12, lines 58-67; col. 13, lines 1-19; col. 34, lines 53-67; col. 35, lines 1-20). Kool et al. teach that polymerases useful in the amplification process include Klenow fragment of DNA polymerase I and AMV reverse transcriptase (col. 13, lines 62-67). Therefore, by teaching amplification of circular RNA targets using these two polymerase Navarro et al. inherently teach formation of multimeric copies of the single-stranded circles.)

Regarding claims 5-8, Navarro et al. teach hexamers and 26-mers (Fig. 1; page 60, fifth and sixth paragraph).

Regarding claims 14 and 58, Navarro et al. teach single-stranded RNA circles (page 59, first paragraph).

Regarding claims 20, 22 and 23, Navarro et al. teach RNA targets in the range of 246-357 nucleotides (page 59, first paragraph), anticipating the limitations.

Regarding claim 25, Navarro et al. teach unknown sequence composition of targets (page 60, first paragraph; page 64, second and third paragraph).

Regarding claims 27 and 35, Navarro et al. teach making radioactively-labeled probes (page 65, first paragraph), therefore they inherently teach radiolabeled (= modified) nucleotides.

Regarding claim 38, Navarro et al. teach primers not resistant to exonuclease activity (page 60, fifth and sixth paragraph).

Regarding claim 51, Navarro et al. teach Klenow fragment of DNA polymerase I (Fig. 1; page 60, last paragraph; page 61, first paragraph).

Regarding claims 55, 57 and 58, Navarro et al. teach AMV reverse transcriptase (Fig. 1). As evidenced by Oyama et al., the AMV reverse transcriptase does not have the 3'->5' exonuclease activity (Abstract; page 448, second paragraph).

Regarding claim 56, Navarro et al. teach Taq DNA polymerase (Fig. 1).

Regarding claim 69, Navarro et al. teach isothermal conditions (Fig. 1).

Regarding claim 70, Navarro et al. teach simultaneous hybridization of the primers to ATC (Fig. 1).

Regarding claim 83, Navarro et al. teach labeling with an intercalator (Fig. 2, 3).

Claim Rejections - 35 USC § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Rejections based on the Navarro et al. reference

10. Claim 9 is rejected under 35 U.S.C. 103(a) as being unpatentable over Navarro et al. (J. Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action), as evidenced by Kool (U.S. Patent No. 6,096,880 A; cited in the IDS) and Oyama et al. (Anal. Biochem., vol. 172, pp. 444-450, 1988).

Navarro et al. teach using hexamer primers, but do not teach octamers.

However, it would have been obvious to one of ordinary skill in the art to have used any primer length appropriate for the experiment, as noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific primer length was other than routine, that the products resulting from the use of specific primer lengths have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

11. Claims 29, 38, 39, 41, 42, 44-47 and 59 are rejected under 35 U.S.C. 103(a) as being unpatentable over Navarro et al. (J. Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action), as evidenced by Kool (U.S. Patent No. 6,096,880 A; cited in the IDS) and Oyama

et al. (Anal. Biochem., vol. 172, pp. 444-450, 1988), and Skerra (Nucl. Acids Res., vol. 20, pp. 3551-3554, 1992; cited in the IDS and in the previous office action).

A) The teachings of Navarro et al. are presented above. Navarro et al. teach Klenow fragment DNA of polymerase I and DNA polymerase I (Fig. 1), which have the 3'->5' exonuclease activity. Navarro et al. do not teach using exonuclease-resistant primers or primers with phosphorothioate nucleotides.

B) Skerra teaches that incorporation of phosphorothioate nucleotides into primers protects them from degradation by the 3'->5' exonuclease activity of DNA polymerases (Abstract; page 3553, last paragraph).

Regarding claims 29 and 44-47, Skerra teaches incorporation of phoshorothioate nucleotides into primers at the 3'-end, making them resistant to exonuclease activity (page 3552, fifth paragraph).

Regarding claims 38, 39 and 59, Skerra teaches mixtures of primers resistant to exonuclease activity and not resistant to exonuclease activity and primers resistant to exonuclease activity (page 3553, paragraphs 1-5).

Regarding claims 41 and 42, Skerra teaches 3'->5' exonuclease activity due to DNA polymerase (page 3551, second paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the exonuclease-resistant primers of Skerra in the method of Navarro et al. The motivation to do so, is provided by Skerra (page 3553, last paragraph):

"The results described here clearly demonstrate that the proofreading activity of thermostable DNA polymerases can severely impair with the correct functioning of primers in the amplification of a DNA sequence. This may be the case either by lowering the yield of the PCR product, in some cases even down to no PCR product at all, or by causing non-specific side

products resulting from 3' terminal editing of the primer molecule. Both effects can be completely avoided by the introduction of a single phosphorothioate bond at the very 3' terminus of the primer which seems to effectively protect the oligodeoxynucleotide from 3' terminal exonucleolytic attack."

Therefore, one of ordinary skill in the art faced with the teaching of Skerra related to detrimental effects of 3'->5' exonuclease activity on the primer integrity would be motivated to use exonuclease-resistant primers in the method of Navarro et al., since the amount of starting material in that method was quite limited, therefore improving priming efficiency would increase efficiency of target amplification.

12. Claims 35, 38, 44, 48 and 49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Navarro et al. (J. Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action), as evidenced by Kool (U.S. Patent No. 6,096,880 A; cited in the IDS) and Oyama et al. (Anal. Biochem., vol. 172, pp. 444-450, 1988), and Ott et al. (Biochemistry, vol. 26, pp. 8237-8241, 1987).

A) The teachings of Navarro et al. are presented above. Navarro et al. teach DNA polymerase I, T4 DNA polymerase and Taq DNA polymerase (Fig. 1), all of which have the 5'->3' exonuclease activity. Navarro et al. do not teach using exonuclease-resistant primers or primers with phosphorothioate nucleotides.

B) Ott et al. teach protection of oligonucleotide primers from the 5'->3' exonuclease activity of a polymerase (Abstract).

Regarding claims 35, 38, 44 and 49, Ott et al. teach incorporation of phosphorothioate nucleotides into 5'-ends of the primers to make the exonuclease resistant (page 8237, second and third paragraph; page 8240, fourth and fifth paragraph).

Regarding claim 48, Ott et al. teach incorporation of more than one phosphorothioate nucleotides into the primer (page 8237, third paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the phosphorothioate nucleotides of Ott et al. in the primers of Navarro et al. The motivation to do, which is provided by Ott et al., would have been that such modification significantly reduces primer degradation by the 5'->3' exonuclease function of the polymerase (page 8240, fifth paragraph). Therefore, faced with the fact that primers annealing around the target RNA molecule would be subject to the 5'->3 exonuclease action of the advancing polymerase (see Fig. 1), one of ordinary skill in the art would be motivated to provide protection from such action according to Ott et al.

Rejections based on Kool and Navarro et al. references

13. Claims 1, 5-9, 14, 20, 22-25, 27, 35, 38, 51, 55-58, 69-71, 75 and 83 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kool (U.S. Patent No. 6,096,880 A; cited in the IDS) and Navarro et al. (J. Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action), as evidenced by Oyama et al. (Anal. Biochem., vol. 172, pp. 444-450, 1988).

A) Regarding claim 1, Kool teaches amplification of short circular DNAs using a primer, dNTPs and a DNA polymerase and producing multiple copies of the circular target by rolling circle synthesis (Fig. 1; col. 5, lines 51-67; col. 6, lines 1-33; col. 12, lines 58-67; col. 13, lines 1-19; col. 34, lines 53-67; col. 35, lines 1-20).

Regarding claims 5-9, Kool teaches primers in the range of 4-50 nucleotides (col. 5, lines 62-64).

Regarding claims 14 and 58, Kool teaches RNA templates (col. 5, lines 59-61).

Regarding claims 20, 22 and 23, Kool teaches circular templates in the range of 15-1500 nucleotides (col. 5, lines 54-57).

Regarding claim 24, Kool teaches bacteriophage DNA (col. 34, lines 55-58).

Regarding claims 27 and 35, Kool teaches radiolabeled nucleotides (col. 13, lines 50-56), therefore inherently teaches modified nucleotides.

Regarding claim 51, Kool teaches Klenow fragment of DNA polymerase I (col. 13, line 65).

Regarding claims 55 and 56, Kool teaches Taq DNA polymerase (col. 13, lines 66, 67).

Regarding claims 57 and 58, Kool teaches AMV and MuLV reverse transcriptases (col. 13, line 67).

Regarding claim 69, Kool teaches isothermic conditions (col. 13, lines 40-49).

Regarding claims 71 and 75, Kool teaches fluorescently-labeled nucleotides (col. 14, lines 25-31).

B) Kool does not teach using multiple degenerate primers.

C) Regarding claim 1, Navarro et al. teach a method of amplification comprising contacting multiple single stranded non-circular degenerate oligonucleotide primers (P1), one or more single stranded amplification target circles (ATCs), a DNA polymerase and multiple deoxynucleoside triphosphates (dNTPs), under conditions promoting said contacting, wherein each ATC hybridizes to a plurality of said P1 primers, wherein said conditions promote rolling circle replication of said amplification target circle by extension of the P1 primers to form multiple tandem sequence DNA (TS-DNA) products and wherein the TS-DNA is labeled during or following synthesis (Navarro et al. teach contacting multiple hexamer random (= degenerate) primers or degenerate 26 bp primers with a single stranded RNA circles in a presence of a DNA

polymerase (AMV reverse transcriptase or Klenow fragment of DNA polymerase I) and multiple dNTPs under conditions promoting rolling circle replication by extension of the primers to form multiple TS-DNA products and wherein the TS-DNA is labeled following synthesis (Fig. 1; page 59, first paragraph; page 60, paragraphs 3-6; page 61, first paragraph; Fig. 2). Navarro et al. do not specifically teach that TS-DNA is obtained in the first step of the viral circles amplification. However, as evidenced by Kool, amplification of small circular DNA or RNA molecules in the range of 15 to 15,000 nucleotides in the presence of a primer and a DNA polymerase results in production of multiple copies of the circular target by rolling circle synthesis (Fig. 1; col. 5, lines 51-67; col. 6, lines 1-33; col. 12, lines 58-67; col. 13, lines 1-19; col. 34, lines 53-67; col. 35, lines 1-20). Kool et al. teach that polymerases useful in the amplification process include Klenow fragment of DNA polymerase I and AMV reverse transcriptase (col. 13, lines 62-67). Therefore, by teaching amplification of circular RNA targets using these two polymerase Navarro et al. inherently teach formation of multimeric copies of the single-stranded circles.)

Regarding claims 5-8, Navarro et al. teach hexamers and 26-mers (Fig. 1; page 60, fifth and sixth paragraph).

Regarding claims 14 and 58, Navarro et al. teach single-stranded RNA circles (page 59, first paragraph).

Regarding claims 20, 22 and 23, Navarro et al. teach RNA targets in the range of 246-357 nucleotides (page 59, first paragraph), anticipating the limitations.

Regarding claim 25, Navarro et al. teach unknown sequence composition of targets (page 60, first paragraph; page 64, second and third paragraph).

Regarding claims 27 and 35, Navarro et al. teach making radioactively-labeled probes (page 65, first paragraph), therefore they inherently teach radiolabeled (= modified) nucleotides.

Regarding claim 38, Navarro et al. teach primers not resistant to exonuclease activity (page 60, fifth and sixth paragraph).

Regarding claim 51, Navarro et al. teach Klenow fragment of DNA polymerase I (Fig. 1; page 60, last paragraph; page 61, first paragraph).

Regarding claims 55, 57 and 58, Navarro et al. teach AMV reverse transcriptase (Fig. 1). As evidenced by Oyama et al., the AMV reverse transcriptase does not have the 3'->5' exonuclease activity (Abstract; page 448, second paragraph).

Regarding claim 56, Navarro et al. teach Taq DNA polymerase (Fig. 1).

Regarding claim 69, Navarro et al. teach isothermal conditions (Fig. 1).

Regarding claim 70, Navarro et al. teach simultaneous hybridization of the primers to ATC (Fig. 1).

Regarding claim 83, Navarro et al. teach labeling with an intercalator (Fig. 2, 3).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used multiple degenerate primers of Navarro et al. in the method of amplification of circular nucleic acid targets of Kool with a reasonable expectation of success. The motivation to do so, provided by Navarro et al., would have been that using degenerate primers allowed amplification of targets with unknown sequences and their cloning using minimum amounts of starting RNA (page 60, second paragraph). Further, as the conditions used by Navarro et al. are conditions which promote rolling circle amplification, as evidenced by Kool, the presence of amplified sequences in the method of Navarro et al. indicates that using multiple primers was not detrimental to the amplification process.

The motivation to use rolling circle synthesis in the amplification of circular targets such as the one of Navarro et al. is provided by Kool (col. 8, lines 41-67):

"The rolling circle method is advantageous for many reasons including the following: (1) it allows optimum production of single-stranded oligonucleotides, unlike PCR and cloning; (2) it uses lower amounts of nucleotide units in the synthesis as compared to DNA synthesizers; (3) it requires only a catalytic amount of circular template and, optionally, primer (PCR to produce DNA oligomers requires stoichiometric amounts of primer); (4) it produces oligomers having clean, well-defined ends (unlike runoff transcription); (5) it is more efficient than single-stranded PCR amplification or runoff transcription because the polymerase enzyme is not required to associate and dissociate from the template in cycles; (6) expensive thermal cyclers and thermostable polymerases are not required; (7) it is possible to make DNA and RNA oligomers and analogs by this method using the same templates; (8) it is better suited for synthesis of circular oligonucleotides; (9) it allows for production in very large batches (hundreds or thousands of grams); (10) it does not use organic solvents or potentially toxic reagents; (11) fewer errors in the sequences are made (machine-synthesized DNA contains structural errors about every 50-100 bases or so, whereas enzyme methods make errors at the rate of about 1 in 10.sup.4 -10.sup.8 bases); and (12) the product generally needs relatively little purification (perhaps gel filtration or dialysis) because only small amounts of template and polymerase are needed to produce large amounts of oligomer."

Therefore one of ordinary skill in the art would have been motivated to combine the methods of Kool and Navarro et al. for the purpose of amplification of unknown circular target sequences from small amounts of starting material for reasons stated by Kool and Navarro et al.

14. Claims 29, 35-39, 44, 45, 48, 49 and 51-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kool (U.S. Patent No. 6,096,880 A; cited in the IDS) and Navarro et al. (J. Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action) (as evidenced by

Oyama et al. (Anal. Biochem., vol. 172, pp. 444-450, 1988)), as applied to claim 1 above, and further in view of Lizardi (5,854,033 A; cited in the previous office action) and Blanco et al. (J. Biol. Chem., vol. 264, pp. 8935-8940, 1989; cited in the IDS).

A) The teachings of Kool and Navarro et al. are presented above. They do not teach exonuclease-resistant primers or phoshorothioate nucleotides, or Φ29 DNA polymerase.

B) Lizardi teaches rolling circle amplification of circular targets (Abstract; col. 2, lines 48-67; col. 3, lines 1-7).

Regarding claims 29 and 35, Lizardi teaches phophorothioate nucleotides (=modified nucleotides) in the amplification (col. 10, lines 24-28; col. 13, lines 27-31).

Regarding claims 36 and 37, Lizardi teaches oligonucleotides attached to solid support, including glass (col. 14, lines 34-43, 65-67; col. 15, lines 1-10).

Regarding claims 38 and 39, Lizardi teaches primers which include modified nucleotides to make them exonuclease-resistant (col. 10, lines 24-28; col. 13, lines 27-31).

Regarding claims 44 and 45, Lizardi teaches that phophorothioate nucleotides are positioned at the 5'-end of the primer to make it exonuclease-resistant (col. 10, lines 24-28; col. 13, lines 27-31). Therefore Lizardi anticipates the limitations of an exonuclease-resistant primer containing at least one nucleotide which makes it resistant to exonuclease activity, a modified nucleotide and a phosphorothioate nucleotide.

Regarding claim 48, Lizardi teaches three or four phosphorothioate nucleotides (col. 10, lines 24-28; col. 13, lines 27-31).

Regarding claim 49, Lizardi teaches the phosphorothioate nucleotides being at the 5' end of the primer (col. 10, lines 24-28; col. 13, lines 27-31).

Regarding claims 51 and 52, Lizardi teaches the following DNA polymerases to be used: bacteriophage ϕ 29 DNA polymerase, phage M2 DNA polymerase, VENT DNA polymerase, Klenow fragment of DNA polymerase I, T5 DNA polymerase, PRD1 DNA polymerase, T4 DNA polymerase holoenzyme (col. 17, lines 66-67, col. 18, lines 1-11). Therefore, since the claim language links 3', 5'-exonuclease activity with these enzymes, and Lizardi specifically teaches them, Lizardi inherently teaches polymerases with 3' -> 5' exonuclase activity.

Regarding claim 53, Lizardi teaches bacteriophage ϕ 29 DNA polymerase (col. 17, lines 66-67, col. 18, lines 1-11) and exonuclease-resistant primers (col. 10, lines 24-28; col. 13, lines 27-31).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the phosphorothioate nucleotides of Lizardi in the primers of Kool and Navarro et al. The motivation to do so, provided by Lizardi, would have been that using primers with such nucleotides prevents their degradation by exonucleases (col. 10, lines 24-27). Since most polymerases cited by Lizardi as suitable for the method have 5' -> 3' exonuclease activity, inclusion of the exonuclease-protective nucleotides in the primers prevented their degradation as well as the degradation of amplification products.

It would have been *prima facie* obvious to have used the ϕ 29 DNA polymerase of Lizardi in the method of rolling circle amplification of Kool and Navarro et al. The motivation to do so, provided by Blanco et al. , would have been that bacteriophage ϕ 29 DNA polymerase was highly processive and did not require any other proteins for efficient rolling circle synthesis of fragments greater than 70 kilobases (Abstract).

15. Claims 29, 35, 38, 39, 41, 42, 44-47 and 59 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kool (U.S. Patent No. 6,096,880 A; cited in the IDS) and Navarro et al. (J.

Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action) (as evidenced by Oyama et al. (Anal. Biochem., vol. 172, pp. 444-450, 1988)), as applied to claim 1 above, and further in view of Skerra (Nucl. Acids Res., vol. 20, pp. 3551-3554, 1992; cited in the IDS and in the previous office action).

A) The teachings of Kool and Navarro et al. are presented above. Navarro et al. and Kool teach Klenow fragment DNA of polymerase I and DNA polymerase I (Fig. 1), which have the 3'->5' exonuclease activity. None of the references teaches using 3’->5’ exonuclease-resistant primers with phosphorothioate nucleotides.

B) Skerra teaches that incorporation of phosphorothioate nucleotides into primers protects them from degradation by the 3’->5’ exonuclease activity of DNA polymerase (Abstract; page 3553, last paragraph).

Regarding claims 29, 35 and 44-47, Skerra teaches incorporation of phoshorothioate nucleotides into primers at the 3’-end, making them resistant to exonuclease activity (page 3552, fifth paragraph).

Regarding claims 38, 39 and 59, Skerra teaches mixtures of primers resistant to exonuclease activity and not resistant to exonuclease activity and primers resistant to exonuclease activity (page 3553, paragraphs 1-5).

Regarding claims 41 and 42, Skerra teaches 3’->5’ exonuclease activity due to DNA polymerase (page 3551, second paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the 3’->5’ exonuclease-resistant primers of Skerra in the method of Kool and Navarro et al. The motivation to do so is provided by Skerra (page 3553, last paragraph):

"The results described here clearly demonstrate that the proofreading activity of thermostable DNA polymerases can severely impair with the correct functioning of primers in the amplification of a DNA sequence. This may be the case either by lowering the yield of the PCR product, in some cases even down to no PCR product at all, or by causing non-specific side products resulting from 3' terminal editing of the primer molecule. Both effects can be completely avoided by the introduction of a single phosphorothioate bond at the very 3' terminus of the primer which seems to effectively protect the oligodeoxynucleotide from 3' terminal exonucleolytic attack."

Therefore, one of ordinary skill in the art faced with the teaching of Skerra related to detrimental effects of 3'->5' exonuclease activity on the primer integrity would be motivated to use exonuclease-resistant primers in the method of Kool, Navarro et al. and Lizardi, since in case when the amount of starting material is limited improving priming efficiency would increase efficiency of target amplification.

16. Claims 72 and 73 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kool (U.S. Patent No. 6,096,880 A; cited in the IDS) and Navarro et al. (J. Virol. Meth., vol. 56, pp. 59-66, 1996; cited in the previous office action) (as evidenced by Oyama et al. (Anal. Biochem., vol. 172, pp. 444-450, 1988)), as applied to claims 1 and 71 above, and further in view of Waggoner et al. (U.S. Patent No. 5,268,486 A; cited in the previous office action).

A) The teachings of Kool and Navarro et al. are described above. Kool teaches fluorescent labels, but do not teach cyanine dyes.

B) Regarding claims 72 and 73, Waggoner et al. teach cyanine fluorescent dyes (Abstract; col. 13, 14).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the cyanine dyes of Waggoner et al. in the method of labeling TS-DNA of Kool and Navarro et al. The motivation to do so, provided by Waggoner et al., would

have been that the dyes were photostable, had high extinction coefficients and high quantum yields (col. 6, lines 11-24).

17. No claims are allowed.

Conclusion

18. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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